The crucial role of leucine concentration on spray dried mannitol-leucine as a single carrier to enhance the aerosolization performance of Albuterol sulfate

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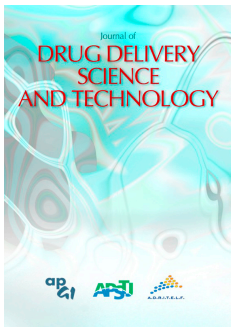

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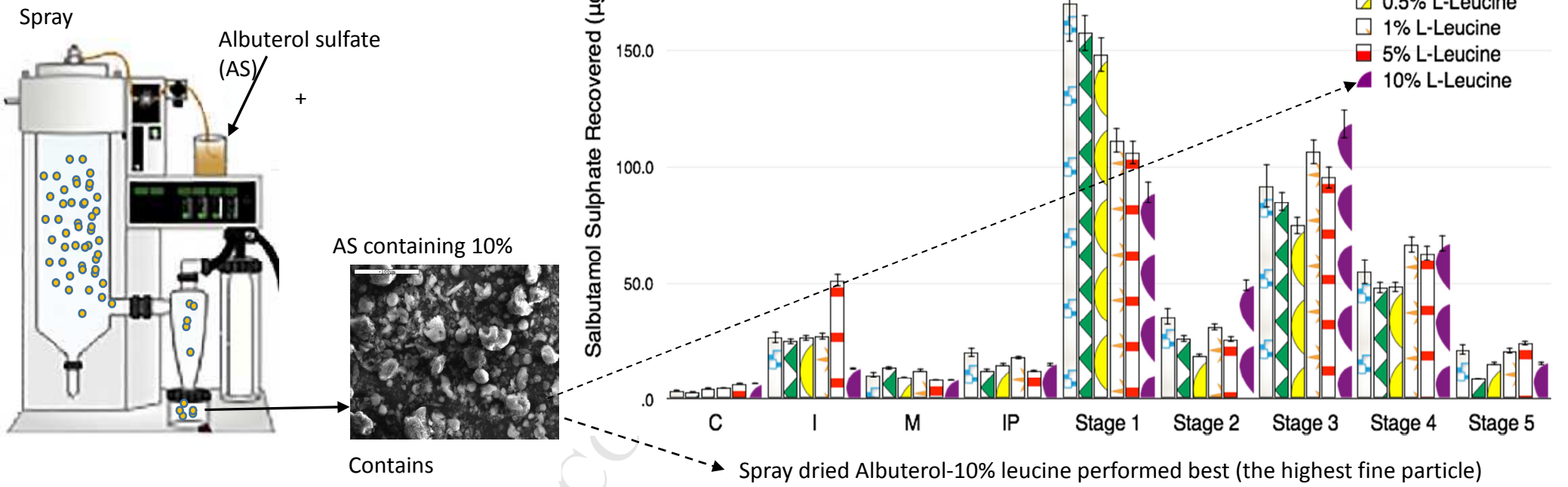
Spray
Albuterol sulfate
(AS)
+
AS containing 10%
Contains
Salbutamol Sulphate Recovered (µg)
200.0
150.0
100.0
50.0
.0
C
I
M
IP
Stage 1
Stage 2
Stage 3
Stage 4
Stage 5
Spray Dried Mannitol
0.1% L-Leucine
0.5% L-Leucine
1% L-Leucine
5% L-Leucine
10% L-Leucine
Spray dried Albuterol-10% leucine performed best (the highest fine particle)

The crucial role of leucine concentration on spray dried mannitol-leucine as a single carrier to enhance the aerosolization performance of Albuterol sulfate

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Abstract

Generally, DPI formulations show low fine particle fraction (FPF) due to poor detachment of drug particles from carrier during inhalation. L-Leucine, with varying concentrations (ranging from 0 to 10% w/w), were introduced into a 60%w/v mannitol solution where the solutions were then spray dried to achieve a new processed carrier. The spray dried samples were blended with Albuterol sulfate to determine the efficacy of their aerosolization performance. Analyzing each formulation was completed via the implementation of numerous analytical techniques such as particle size distribution analysis via laser diffraction, differential scanning calorimetry (DSC), scanning electron microscope (SEM), powder X-Ray diffraction (PXRD), Fourier transform infrared (FT-IR) spectroscopy, and an *in vitro* deposition study. It was shown the concentration of leucine in spray dried is really crucial to achieve the highest FPF possible. The highest FPF was obtained for the samples containing 10% w/w leucine which was 52.96±5.21%. It was interesting to note that the presence of leucine produced different polymorphic forms for mannitol. Moreover, through this study, the authors were able to conclude that mannitol can serve as an alternative carrier in DPI formulations containing Albuterol sulfate tailored for lactose intolerant patients.

Keywords: Spray dried mannitol-Leucine; Albuterol sulfate; polymorphic form; dry powder inhaler; aerosolization behaviour; fine particle fraction

1. Introduction

Dry powder inhalers (DPI) are a common tool for use in patients facing chronic obstructive pulmonary disease (COPD) and asthma. It has previously been documented that pulmonary delivery of a therapeutic dose has tremendous advantages over other administrative routes [1]. In recent years, the respiratory tract has been used as a diagnostic tool for patients suffering from intermittent allergic asthma or allergic rhinitis through the use of mannitol as a means to increase the water content in the respiratory tract [2]. It has also been postulated that mannitol could be an alternative carrier in DPI formulations to lactose [3], which is a carrier that is widely used in the pharmaceutical industry [4].

While an increase in the use of DPIs has been seen through recent years, there still exists a major setback in their effort to deliver a consistent therapeutic dose to each patient [5] while also having poor aerosolization performance. Efforts have been employed to tackle such problem by focusing on physicochemical properties associated with each of the carriers such that an increase in the efficacy of DPI aerosolization performance was recorded [6].

It has been shown that mannitol has an altering effect on the viscoelastic properties associated to the phlegm located in the airway while also increasing its water content by creating an osmotic gradient which facilitates an efflux of water into the airway lumen [7-13]. In addition, mannitol is not classified as being a reducing sugar, given the absence of the aldehyde functional group, and it is less hygroscopic than lactose while providing a sweet aftertaste which can be used as a benefit for the patient by confirming that an adequate dose has been delivered [14, 15].

Spray drying provides an avenue by which physicochemical properties could be modified in such a way as to yield a formulation suitable for pulmonary delivery via inhalation [1, 16-19]. Coupling the advantages of both the spray drying technique with that of mannitol and the results obtained from Molina *et al* [6] where $_{L}$-Leucine was used as an excipient to determine its effect on DPI formulations provides the fundamental groundwork for this study. Although, the previous study showed that the presence of leucine, as an additive, can improve the aerosolization performance of salbutamol

sulfate containing lactose [6], in the case of lactose intolerant patients the formulation containing lactose would be under question. Mannitol could be an alternative to lactose to be used in DPI formulation as a carrier, but the commercial mannitol shows a poor performance as a carrier in DPI formulations [3]. Therefore, the aim of the current research is to explore whether spray dried mannitol-leucine could be a good alternative in DPI formulations. Unfortunately, the results of lactose cannot be extended to mannitol as the type of carrier can change the concentration of the leucine needed to reach the optimum aerosolization performance hence the optimum FPF. On the basis of the above explanation, the current research engineers mannitol particles in the presence of leucine to achieve desirable and specific morphologies such that optimal carrier conditions were met. Engineered carriers will be used with Albuterol sulfate, with efforts to increase the efficiency of the DPI performance. In addition, the effect of spray drying on particulates and their role in the engineered DPI formulations were investigated.

2. Materials and Methods

2.1 Materials

Mannitol (Pearlitol) was supplied from Roquette (Lestrem, France; 99.9% pure), Albuterol sulfate from L.B. (Bohle, Germany; 99.9% pure), and $_{L}$-Leucine by Acros Organics (Geel, Belgium; 99.9% pure). Monobasic potassium phosphate (Geel, Belgium; 99.9% pure) was used for the preparation of the mobile phase for high-pressure liquid chromatography (HPLC). Methanol, ethanol, and hydrochloric acid were purchased from VWR International Ltd. (Leighton Buzzard, United Kingdom) and were HPLC grade.

2.2 Spray Drying

Spray drying was conducted using the Mini Spray Dryer B-290 from Buchi (Flawil, Switzerland) equipped with a dehumidifier (Dehumidifier B-296), an inert loop (Inert Loop B-295), and an outlet filter in a closed system with the use of nitrogen gas (N_2). Parameters associated were those that are outlined in detail elsewhere [6] where several rigorous optimization procedures were implemented to achieve the selected parameters and overall protocol. In brief, the parameters associated with the procedure were as follows: inlet temperature of 220 °C, aspirator set to 100%, pump rate set to 5%, and a flow rate of 22%.

Each 100 mL of spray dried solution contained different concentrations of $_{L}$-Leucine (0.0, 0.06, 0.3, 0.6, 3.0, and 6.0 g) and $_{D}$-Mannitol (60.0, 59.94, 59.7, 59.4, 57.0, and 54.0g; respectively). Meaning that the percentage of $_{L}$-Leucine in each solution was 0.0, 0.1, 0.5,1.0, 5.0, and 10.0% w/w, respectively. Both $_{L}$-Leucine and $_{D}$-Mannitol were dissolved in double distilled water and were heated to 75 °C with a stirring speed of 120rpm; the final solutions were then spray dried under the conditions mentioned above.

2.3 Sieving

Sieving was conducted to remove coarse particles from smaller particles that would interact negatively against Albuterol sulfate [19]. Thus, collection of particles within 63-90 μm size range was done using a Retsch AS 200 Digit Analytical Sieve Shaker (Hoan, Germany) where sieving was performed for 30 minutes with an amplitude of 100 for each of the carriers prior to particle analysis. The range of 63-90 μm was selected because it follows the guidelines set forth by the USA Pharmacopoeia [21]. Furthermore, particles that fell within the range of 63-90 μm were collected, sealed, and stored in glass vials in an air-conditioned laboratory with a set temperature of 20 ºC and a relative humidity (RH) of 50% for future use within this study.

2.4 Particle Size Distribution Analysis

Particle size distribution analysis was conducted using a laser diffraction particle size analyzer (Sympatec Ltd., Germany) equipped with a HELOS sensor and Windox software. Analysis of the carriers was completed using both the Rodos (dry system) and Cuvette (wet system); the cuvette system required the use of absolute ethanol and a stirring speed of 1200rpm while the Rodos system required a pressure of 3.0 bar, feed rate of 60%, and trigger conditions that used optical concentration of greater than or equal to 0.2%. Detecting the particles was done using the R3 and R5 lenses, which have a particle size detection range of 0.5-175μm and 0.5-875μm, respectively.

The span of size distribution was calculated using Equation 1 where $D_{90\%}$, $D_{50\%}$, and $D_{10\%}$ refer to the particle size (in μm) of 90, 50, and 10% of the cumulative particle size distribution, respectively. The aerodynamic diameter was calculated using Equation 2 where d_{aer} refers to the aerodynamic diameter, d_g to the geometric diameter, P to the density of the particle, P_0 to the unit density, and X to the shape factor [22, 23].

$$Span = \frac{D90\% - D10\%}{D50\%} \quad \text{(Eq. 1)}$$

$$d_{aer} = d_g \sqrt{P/_{\chi * P_0}} \quad \text{(Eq. 2)}$$

2.5 Preparation of Dry Powder Inhalation (DPI) formulations

Using the stored 63-90 μm sieved carriers, Albuterol sulfate (AS) was introduced such that a final ratio of carrier: SS was 67.5:1. This ratio corresponded to a theoretical dosage of 482 ± 1.5 μg of SS per single unit. To this end, 1.35 g of each carrier and 20 mg of SS were mixed. Mixing was carried out with the use of a Turbula Type T2F (Junkermattstrasse, Switzerland) where each of the formulations was subjected to 30 minutes of mixing at a speed of 72 rpm ensuring each formulation was thoroughly blended.

2.6 Differential Scanning Calorimetry Analysis

Perkin Elmer's (Shelton, Connecticut, United States of America) differential scanning calorimetry (DSC) 4000 equipped with a standard single-furnace was used to perform thermodynamic analysis; viewing and analyzing the data was completed with the accompanied Pyris Series software. Each sample was accurately weighed, where the mass ranged from 4-5 mg per sample, on aluminium pans and sealed with an aluminium cap. The DSC was calibrated using indium and zinc prior to any analysis. Samples were scanned from 25 to 320 ºC with a scanning rate of 10 ºC/min.

2.7 Powder X-Ray Diffraction (PXRD)

PXRD was performed by implementing Siemens' Diffractometer D5000 (Munich, Germany), where 200 mg of each sieved carrier was placed on a holder such that a leveled surface was obtained when observed in comparison to the pan and diffractometer. Prior to analysis, the holder was placed on the Diffractometer in a manner where analysis at a specific angle was possible. At which point, the sample was exposed to X-Rays (Cu K_α- 1.54056Å) with a voltage of 40 kV and a current of 30 mA while being scanned from 5-50° on the 2θ plane at a scanning rate of 0.1 2θ increments per second.

2.8 Fourier Transform Infrared (FT-IR) Spectroscopy

In order to assess any changes in the molecular level of the engineered particles, [24]. FT-IR was used (Perkin Elmer's Spectrum One, Shelton, Connecticut, United States of America) equipped with a Universal ATR). Preceding to analysis, methanol was used to clean the instrument to remove any residual matter left on the apparatus, after which a few milligrams of each of the carries was used with a pressure of 100 bar. Each of the samples was scanned three times over a range of 4000 cm^{-1} to 500 cm^{-1} to obtain spectra with appropriate resolution.

2.9 Scanning Electron Microscope (SEM)

Electron micrographs were obtained using a JMS-820 Scanning Microscope (Freising, Germany) with a voltage of 4 kV, to evaluate particle morphology, size, shape, and presence or absence of agglomerates. Before subjecting each carrier to electrons, they were thinly placed on double-sided carbon tape followed by coating with gold (Au) for 5 minutes under a vacuum in an Argon-rich environment; to view each of the carriers different magnifications were employed.

2.10 Homogeneity Assessment

In the homogeneity test, 5 samples from each of the formulations, which yielded a mass range of 50 mg (this will give absorbance around 0.6), were introduced to 50 mL of double distilled water in volumetric flasks for analysis by UV-VIS spectroscopy; the wavelength associated to the assay was set at 225 nm (mannitol and leucine have no absorbance in this wavelength). Results are based on obtaining the average of the five samples which also includes the standard deviation for each distinct formulation.

2.11 Deposition Study

A Multi-Stage Liquid Impinger (MSLI), equipped with a USP induction port (Copley Scientific in Nottingham, United Kingdom), was used alongside the Critical Flow Controller (Copley TPK) and a High Capacity Pump (Copley HCP5) that allow for a 4 kPa flow rate drop to be observed. Moreover, Equation 3 was employed to determine the test flow duration (in seconds) used within each deposition to adhere with the United States Pharmacopeia (USP) specific standard test methods for Aerosols, Nasal Sprays, Metered-dose inhalers (MDIs), and Dry Powder Inhalers [21].

$$T = \frac{240}{Q_{out}} \quad \text{(Eq. 3)}$$

where Q_{out} is the volume of air passing through the airflow meter.

Each deposition study used 10 capsules per run, where every capsule was filled with 33.19±0.13 mg of the Carrier:SS being investigated which corresponded to a theoretical API dose of 482±1.5 μg of Albuterol sulfate per capsule. All of the formulations were tested a total of three times, equivalent to 30 capsules per formulation.

In addition, specific parameters were employed for the analysis of the aerosolization of the capsules including the recovery dose (RD), emitted dose (ED), percent recovery, percent emission, impaction loss, mass median aerodynamic diameter (MMAD), geometric standard deviation (GSD), fine particle fraction (FPF), fine particle dose (FPD), drug loss (DL), dispersibility (DS), and effective inhalation index (EI).

RD is defined as the amount of drug (in μg) recovered from the inhaler, induction port (IP), mouthpiece (M), and stages 1-5 (S1-5), ED as the amount of drug (in μg) recovered from IP and S1-5, percent recovery as the ratio of RD to the theoretical dose (482±1.5 μg), percent emission as the ratio of ED to RD, impaction loss as the mass fraction of drug in IP and S1 to RD (IP + S1: RD), MMAD as the logarithmic function of the cut-off diameter to the corresponding concentrations of particles found within each stage of the MSLI, GSD as the square root of the 84^{th} and 15^{th} percentile, FPF as the ratio between FPD to RD (FPD:RD), FPD as the sum of drug (in μg) from S3-5, DL as the ratio of the amount of Albuterol sulfate recovered from capsules, mouthpiece, and inhaler to RD (capsules + (I + M)): RD), and DS as the ratio of FPD to ED (FPD:ED).

Furthermore, to determine the effective inhalation index (EI) of each of the formulations, Equation 4 was implemented where EI refers to the effective inhalation index, EM to the percent emission, and FPF to the Fine Particle Fraction [25]:

$$EI = \sqrt{(EM + FPF)} \quad \text{(Eq. 4)}$$

All the deposition studies were conducted in an air-conditioned laboratory where the temperature was 20°C and the relative humidity (RH) was 50%.

2.12.1 High Pressure Liquid Chromatography (HPLC)

Qualitative and quantitative analysis of Albuterol sulfate was completed by using the protocol that published elsewhere [6]. Execution of HPLC, however, was completed via the Agilent 1100 Series HPLC System (Santa Clara, California, United States of America) where a degasser (G1322A), binary pump (G1312A), variable wavelength detector (VWD G1314A), column thermostat (G1316A), and thermostatted auto-sampler (ALS G1329A) were coupled with the Waters Spherisorb 5µm ODS2 4.6x150mm Analytical Column (Milford, Massachusetts, United States of America); to analyze and view the chromatographs, *ChemStation* Software was utilized. Likewise, internal standards of varying Albuterol sulfate concentration (0.00. 0.50, 2.50, and 5.00µg/mL, respectively) were used to calibrate and normalize the results.

2.12.2 Determination of leucine by HPLC Method

To quantify leucine concentration in the spray dried samples, a mobile phase containing 50% (v/v) of 0.1% Trifluoroacetic acid (TFA) in water and 50% (v/v) of methanol was used. The flow rate of the mobile phase through the HPLC column was 0.8 mL/min with a total run time of 15 minutes per injection set at a wavelength of 260 nm yielding a retention time of 3 minutes. Calibration standards of varying leucine concentrations (0.00, 0.50, 1.00, 5.00, and 10.00mg/mL, respectively) were used to calibrate and normalize the results.

2.13 Statistical Analysis

One-way analysis of variance (ANOVA) was used to evaluate the results in this study where statistical probability (P) values less than 0.05 were considered a significant difference when using the Tukey's Honestly Significant Difference (HSD) test. Data is expressed as the mean ± standard deviation and the typical number of replicates was n= 3 except in the uniformity test where n was 5.

3. Results and Discussion

3.1Particle Size Analysis

Figure 1 shows the cumulative size distribution obtained by two distinct systems: Rodos (dry system; Figure 1A) and Cuvette (wet system; Figure 1B). The spray drying system used in the current study should provide particle size below 20 μm [26, 27], but Figure 1B showed that all of the carriers underwent some degree of agglomeration. When the wet system (Figure 1B) was changed to the dry system (Figure 1A), due to the application of 3 bar pressure to spray the dry particles, the pressure applied was able to de-agglomerate particles and reduce the size range of spray dried samples, which is an indication of the presence of agglomerated particles in the samples.

Table 1 elucidates the volume mean diameter (VMD) along with the span of each of the distinct carriers when using the Rodos and Cuvette systems comparing them side-by-side. All of the carriers experienced a significant difference in their VMDs ($p < 0.05$) with ranges from 23.98 ± 0.26 μm to 52.99 $\pm$ 4.05 μm for spray dried mannitol and 10% leucine respectively when the dry system was used; in the case of using the wet system, these values increased (Table 1). In all cases, the results showed that VMD for spray dried samples measured by the dry system was smaller than when the wet system was used. Results from Table 1 show that the difference between VMD of these two techniques used to measure the particle size is an indication of particle agglomeration which in the dry system these particles de-agglomerate as a result of applying 3 bars pressure during the measurement of the particle size. This was supported by SEM images in Figure 2. Furthermore, it was concluded that all of the carriers from each of the formulations underwent a degree of agglomeration as the aggregated size of particles were closer to the size of **the carrier** used in DPI formulations. Therefore, it was concluded that the performance of the DPI system was not compromised due to the size of the particles.

With respect to the Span, all of the carriers experienced similar values ($p > 0.05$) having ranges from 1.45 $\pm$ 0.15 belonging to 10% Leu:Mannitol to 2.69 $\pm$ 0.44 from 1%

Leu:Mannitol and 1.08 ± 2.77 from 0.1% Leu:Mannitol to 2.02 ± 0.20 from 5% Leu:Mannitol; for both the dry and wet systems, respectively. Moreover, the dry system experienced a particle diameter range of 5.98 ± 0.76 μm ($D_{10\%}$) to 66.76 ± 1.66 μm ($D_{90\%}$) where the particle diameter range for the wet system fell between 28.77 ± 0.62 μm ($D_{10\%}$) and 124.72±3.62 μm ($D_{90\%}$).

Figure 2, nonetheless, presents the electron micrographs of each of the carriers in each formulation (0, 0.1, 0.5, 1, 5, and 10% leucine; respectively). All of the carriers were characterized as spheroidal with confirmation of there being some degree of agglomeration, which correlates to the results already presented in Figure 1 and Table 1.

3.2 Solid-State Characterization

Figure 3 presents the DSC traces of leucine, spray dried mannitol, spray dried mannitol containing 0.1%, 0.5%, 1%, 5% and 10% leucine. The endothermic peak observed in the DSC traces is associated to the melting of mannitol. Moreover, Table 2 summarizes the enthalpy and melting peak of each formulation.

Table 2 authenticates the results presented in Figure 3, where it illustrates an endothermic event at 169.79 ± 0.45°C which is known to be the melting of mannitol [25, 28] and is an indication of the crystalline nature of the spray dried mannitol containing leucine. An important observation to highlight, nonetheless, is the broadening of the endothermic peak as the leucine concentration increases (Table 3). Given that more energy is required to melt the mannitol crystals, due to leucine being present, explains leucine's ability to increase the enthalpy of the melting that provides a stabilization effect for the carries; the associated bonding energy and the associated reaction mechanism makes this phenomenon possible. Additionally, pure leucine was tested to determine whether or not any thermal event would take place between the 25-300 °C range, and, as can be seen in Figure 3, it was well above 300°C.

Figure 4 demonstrates the powder X-ray diffraction patterns obtained from each of the formulation's carriers highlighting the presence and location of the distinctive polymorphic characterization. It is understood that mannitol possesses three distinctive polymorphs (α-, β-, and δ-) that are characterized by where they present themselves on

the diffraction patterns; with α-mannitol exhibiting peaks at 9.57° and 13.79°, β-mannitol at 10.56° and at 14.71°, and δ-mannitol with a peak at 9.74° and 22.2° [29-32].

XRD of commercial mannitol showed the main diagnostic peaks for β-mannitol at 10.56°, 14.71°, 23.4°, 29.5° and 38.8°. This indicates that the commercial mannitol is only β form of mannitol. Spray Dried Mannitol showed extra peaks at 2θ of 13.79° and around 17° which is an indication of α-mannitol. This shows that the spray dried mannitol contains both α- and β-mannitol. It is obvious from XRD of spray dried mannitol containing various concentrations of Leucine, these samples containing both α- and β-mannitol. Although all spray died samples showed the presence of α- and β-mannitol, the intensity of diagnostic peaks **is** not the same which could be an indication of different ratios of these two polymorphic forms in the sample. The lack of any diagnostic peak at 9.74° and 22.2 indicates there is no delta mannitol in the samples. Table 4 summarizes all the polymorphic forms of mannitol associated with each formulation. The presence of sharp peaks in the XRD patterns is an indication of the crystalline nature of the mannitol-leucine samples.

In a study conducted by Kaialy et al., it was found that freeze-dried mannitol containing the 3 polymorphic forms (α-, β-, and δ-) produced a larger endotherm peak than mannitol with 2 polymorphic forms (α- and β-) [32]. Conventionally, the melting enthalpy (ΔH) represents the degree of crystallinity of a substance. By mixing two substances, the purity is reduced and lower melting points appear in the DSC thermographs. Any shift in melting point is indicative of a strong solid-solid interaction, which explains why the 10% Leucine carrier had the broadest thermal peak [33, 34]. In conclusion, XRD results showed that all formulations are in crystalline state regardless of the type of polymorphic form they contain.

Solid-state characterization was further assessed with the implementation of FT-IR (Figure 5). It was understood that α-mannitol exhibits a peak at 1195 cm^{-1}, β-mannitol at 929 cm^{-1}, 959 cm^{-1}, and 1209 cm^{-1}, and δ-mannitol at 967 cm^{-1} [3]. Looking at Figure 5, it is clear that the commercial mannitol shows the main peaks for beta mannitol. Spray Dried Mannitol exhibited the peaks associated to the β-polymorph (alpha mannitol was not detectable due to a very low concentration, it was more clear in XRD figure) whereas all other spray dried samples containing leucine showed peaks associated to the α-mannitol and β-mannitol polymorphs.

The apparent broadening and widening in the peaks within 2500-3700 cm^{-1} **are** due to the presence of leucine in the samples. Leucine, being a branched-chain amino acid (BCAA), belongs to a group of proteins that are known for having an aliphatic side-chain and that are non-polar; the aliphatic side-chain explains the results obtained in the spectra. In essence, the presence of leucine allowed for there to be an increase in the vibrational stretching that is observed by the hydroxyl group [35].

3.3 Analysis of Formulations

3.3.1 Albuterol Sulfate Assessment

Aerosolization performance of all of the formulations is summarized in Figure 6 where the amount of Albuterol sulfate deposited in each of the stages of the deposition is shown [capsules (C), inhaler (I), mouthpiece (M), induction port (IP), Stage 1, Stage 2, Stage 3, Stage 4, and Stage 5]. All of the formulations experienced minimal Albuterol sulfate deposits ($p > 0.05$) in the capsules with 5% leucine having the highest amount (6.62 $\pm$ 4.59 μg) and 0.1% leucine having the lowest amount (2.98 $\pm$ 0.42 μg). As particles maneuvered through the simulated respiratory tract (MSLI), 5% leucine experienced the highest amount of Albuterol sulfate (51.35 $\pm$ 49.66 μg) in the inhaler when compared to 10% leucine, which experienced the least amount at 13.26$\pm$6.34 μg. Furthermore, all of the formulations showed similar amounts of Albuterol sulfate in the mouthpiece and induction port (see Figure 6), but began to differ at Stage 1 where aerodynamic particle size becomes more significant.

Moreover, Spray Dried Mannitol had the highest Albuterol sulfate recovered from within Stage 1 (170.70 + 37.06μg), but 0.1% leucine and 0.5% leucine were not far behind with 157.75 $\pm$ 9.04 μg and 148.30 $\pm$ 32.12 μg, respectively. This shows that as the concentration of leucine increases the amount of Albuterol sulfate deposits in Stage 1 decreases. This could be due to the lubrication effect that is seen when leucine is added as an excipient. The results showed that 10% leucine experienced the highest Albuterol sulfate amounts from within Stage 3, and Stage 4 (118.74 $\pm$ 44.84 μg, and 67.40 $\pm$ 15.75 μg; respectively) indicative of it being the most successful at delivering Albuterol sulfate to the lower part of the lungs. In other words, the formulations, with respect to MMAD, ranked in the following order: 10% leucine = 0.1% leucine > Spray Dried Mannitol > 1% leucine > 0.5% leucine > 5% leucine.

Likewise, looking at the RD, ED, and percentage recovery of each formulation, which is presented in Table 5, it was concluded that all of the formulations, with the exception of Spray Dried Mannitol, experienced similar values ($p < 0.05$). Such results are indicative of leucine's powder dispersion effect [36-38] and its ability to act as a lubricant, and its ability to aid in storage and stability [39] as Spray Dried Mannitol showed significantly different results and contained no leucine.

Additionally, all of the formulations differed remarkably from one another with respect to drug loss (DL), see Table 5, given that they all undertook a high number of actuations (n= 10) per run with each being filled with a consistent weight of 33.13 $\pm$ 0.46 mg. Nevertheless, 10% leucine experienced the least amount of drug loss with 9.64 $\pm$ 1.01% indicative of optimized properties allowing for the best attachment and detachment of SS when compared to all other formulations.

It is interesting to note that when the concentration of leucine increased from 0 to 0.5% no significant changes **($p>0.05$)** were observed in impaction loss (IL), whereas beyond 0.5% a significant reduction **($p<0.05$)** was observed for the IL value so that samples containing 10% leucine showed the least IL (28.74 $\pm$ 9.13%). Such variation between the formulations could be attributed to their aerodynamic diameter given that impaction is a flow-dependent mechanism governed by particle size [40]. In addition, 10% leucine showed the smallest VMD of its coarse particulate matter (VMD of 63.46 $\pm$ 0.18µm; results from Table 1) when compared to the other formulations; all of which had higher VMDs for their agglomerated coarse particles (see Table 1 and Figure 2).

Effective inhalation index (EI) ranged from 11.28 $\pm$ 0.16 (0.1% leucine) to 12.14 $\pm$ 0.21 (10% leucine) showing a linear relationship with FPF ($r^2 = 0.81$), data not shown. This indicates that the presence of leucine is necessary to enhance the EI value.

DS (dispersibility) and FPD also confirmed that samples with higher concentrations of leucine showed better dispersibility and high fine particle dose where both are an indication of a good aerosolization performance of Albuterol sulfate. There was a linear relationship ($r^2 = 0.89$) between the carriers of the wet system's VMDs and that of FPD

(data not shown) which suggests inertial impaction and its prevalence, as previously discussed; such relationship also builds upon the variations observed between the formulations.

When it came to MMAD and GSD, however, all of the formulations gave similar results with MMAD and GSD ($p > 0.05$). In addition, a linear correlation ($r^2 = 0.69$) between the leucine concentration and FPF was established (data not shown) suggesting that leucine played a significant role ($p < 0.05$) in decreasing the particle's density and size [38], while providing an anti-hygroscopic effect [39], as it has been shown for leucine to precipitate on the surface of drying droplets when spray drying (16, 37, 38, 41, 42] These precipitated leucine patches were accounted for when engineering the carriers as the end product shows; knowing this aids in the developmental process for physicochemical property selection and with the invention/creation of methodological processes.

Furthermore, 10% leucine exhibited the highest FPF of 52.96 $\pm$ 5.21% indicative of it being the most efficient at delivering the highest amount of SS to the lower respiratory tract. In addition, this formulation also showed the best drug-carrier cohesive-adhesive balance ratio as this ratio is directly related to the FPF of any given API [28]. Such results also support those of [43] which experienced a similar outcome. Moreover, this formulation also had the highest percentage emission of 94.35 $\pm$ 0.64%, when compared to the other formulations (see Table 5), inferring that it released the most SS into the system, which then becomes coupled with the aforementioned findings; providing sufficient evidence to classify it as the best formulation.

All in all, optimal properties were attained such that a complementary system emerged between SS and the 10% leucine carrier and one that was effective when implemented. Physicochemical properties, particle size, particle density, and particle morphology were used to attain favourable conditions for the 10% leucine carrier-to-SS system. On the other hand, Spray Dried Mannitol showed the lowest FPF (37.06 $\pm$ 8.66%) inferring that SS had a more difficult time detaching itself from the Spray Dried Mannitol carrier during the inhalation process when compared to 10% $_{L}$-Leucine, which performed with the highest efficacy profile for aerosolization purposes.

3.3.2 Homogeneity Assessment

Assessing the homogeneity of each formulation was an essential phase of this overall study given that a uniform formulation will give rise to a more effective drug delivery profile with a consistent dose to the patient; it also adheres to USP guidelines. Figure 7 shows the homogeneity profile of each of the formulations (0, 0.1, 0.5, 1, 5, and 10% $_{L}$-Leucine) under investigation showing the potency of each while Table 6 presents the percent content homogeneity, which is expressed as the percentage coefficient of variation (%CV), of each of the formulations studied.

All of the formulations varied considerably from one another with regard to potency with a range of 122.37 $\pm$ 4.75% (sample containing 0.1% leucine) to 91.95 $\pm$ 19.22% (Spray Dried Mannitol without leucine). Regarding %CV, the smallest %CV of 3.88% belonged to 0.1% leucine and the highest %CV of 20.90 belonged to Spray Dried Mannitol without leucine (see Table 6). Such results indicate that 0.1% leucine had the best Albuterol sulfate content homogeneity amongst all of the formulations. It is important to mention that the mixing process facilitates the emersion of friction between particle surfaces, via triboelectrification, which can affect substantially the quality of the blend, its homogeneity, and the segregation tendencies as previously mentioned [44, 45]. This could be the main reason that CV% goes above 5%. Table 6 also showed that the presence of leucine improved the homogeneity of the samples compared to the sample without leucine. The table also shows that all formulations adhered to the acceptable range of 75-125% set by the USP.

4. Conclusion

Mannitol solutions containing different concentrations of leucine were successfully spray dried. The results showed that the presence of leucine changed the properties of the resultant spray dried particles. The presence of leucine in spray dried formulations improved the aerosolization performance of Albuterol sulfate where the FPF with 10% leucine appeared to be the highest FPF of 52.96 $\pm$ 5.21%. Through this study, it was also confirmed that mannitol serves as a suitable alternative carrier over lactose in DPI formulations containing leucine and could be suitable for lactose intolerant patients suffering from asthma. In the future, it would be beneficial to explore the use of both spray dried lactose and mannitol, together, to determine their effect when used in DPI

formulations. In addition, determining the surface energies of each carrier can also be beneficial in determining the carrier's overall aerosolization performance.

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Table 1. Particle Analysis of Spray Dried Mannitol, 0.1% L-Leucine, 0.5% L-Leucine, 1% L-Leucine, 5% L-Leucine, and 10% L-Leucine showing the volume mean diameter (VMD) and span when using the Rodos dry system or the Cuvette wet system

Carrier	VMD (µm) Dry System	VMD (µm) Wet System	Span Dry System	Span Wet System
Spray Dried Mannitol	23.98 ± 0.26	69.55 ± 3.85	2.43 ± 0.09	1.65 ± 3.50
0.1% L-Leu:Mannitol	40.50 ± 0.73	81.10 ± 2.34	1.55 ± 0.83	1.08 ± 2.77
0.5% L-Leu:Mannitol	33.91 ± 1.34	87.03 ± 2.54	1.97 ± 0.63	1.09 ± 2.60
1% L-Leu:Mannitol	27.05 ± 0.95	64.74 ± 1.69	2.69 ± 0.44	1.42 ± 2.02
5% L-Leu:Mannitol	29.86 ± 0.19	72.82 ± 0.07	2.25 ± 1.95	2.02 ± 0.20
10% L-Leu:Mannitol	52.99 ± 4.05	63.46 ± 0.18	1.45 ± 0.15	1.63 ± 6.71

Table 2. DSC thermal traces of Spray Dried Mannitol, 0.1% $_{L}$-Leucine, 0.5% $_{L}$-Leucine, 1% $_{L}$-Leucine, 5% $_{L}$-Leucine, 10% $_{L}$-Leucine, and $_{L}$-Leucine that indicate the enthalpy, in J/g, of mannitol melting (ΔH) along with the Temperature (°C) of where such melting took place

Carrier	Temperature (°C)	ΔH (J/g)
Spray Dried Mannitol	170.01 ± 0.16	198.38 ± 8.97
0.1% L-Leucine	170.07 ± 0.77	175.52 ± 55.68
0.5% L-Leucine	170.45 ± 0.11	213.10 ± 6.17
1% L-Leucine	169.49 ± 0.06	182.87 ± 19.44
5% L-Leucine	169.32 ± 0.33	201.98 ± 16.61
10% L-Leucine	169.41 ± 0.15	213.79 ± 3.21
L-Leucine	—	—

Table 3. Actual amount of $_{L}$-Leucine found in each of the carriers (Spray Dried Mannitol, 0.1% $_{L}$-Leucine, 0.5% $_{L}$-Leucine, 1% $_{L}$-Leucine, 5% $_{L}$-Leucine, and 10% $_{L}$-Leucine)

Formulation	% Leucine
10% Leucine	12.93 ± 1.19
5% Leucine	7.24 ± 2.01
1% Leucine	2.86 ± 1.22
0.5% Leucine	0.82 ± 1.84
0.1% Leucine	0.02 ± 0.02
SD Mannitol	0.00 ± 0.00

Table 4. Characterization of mannitol polymorphs found within each formulation's carriers.

Carrier	α-mannitol	β-mannitol	δ-mannitol
Commercial mannitol	---	✓	---
Spray Dried Mannitol	✓	✓	
0.1% $_{L}$-Leucine	✓	✓	---
0.5% $_{L}$-Leucine	✓	✓	---
1% $_{L}$-Leucine	✓	✓	---
5% $_{L}$-Leucine	✓	✓	---
10% $_{L}$-Leucine	✓	✓	---

Table 5. Recovered Dose (RD), Emitted Dose (ED), Percent Recovery, Percent Emission, Percent Impact Loss, Mass Median Aerodynamic Diameter (MMAD), Geometric Standard Deviation (GSD), Fine Particle Dose (FPD), Fine Particle Fraction (FPF), Drug Loss (DL), Dispersibility (DS), and Effective Inhalation Index (EI) of Albuterol sulfate obtained from each of the different formulations (Spray Dried Mannitol, 0.1% $_{L}$-Leucine, 0.5% $_{L}$-Leucine, 1% $_{L}$-Leucine, 5% $_{L}$-Leucine, and 10% $_{L}$-Leucine)

Formulation	RD (µg)	ED (µg)	Recovery (%)	Emission (%)	Impact Loss (%)	MMAD (µm)	GSD (µm)	FPD	FPF (%)	DL (%)	DS (%)	EI
Spray Dried Mannitol	431 ± 143.68	394.56 ± 147.19	89.74 ± 29.87	90.34 ± 5.20	45.59 ± 6.29	3.06 ± 0.10	2.10 ± 0.08	168.20 ± 85.39	37.06 ± 8.66	10.54 ± 5.35	40.75 ± 7.55	11.28 ± 0.62
0.1% L-Leucine	376.84 ± 41.04	338.39 ± 41.00	78.034 ± 8.53	89.72 ± 1.16	45.34 ± 2.08	3.20 ± 0.05	2.01 ± 0.03	142.08 ± 24.68	37.54 ± 2.46	11.08 ± 1.25	41.83 ± 2.19	11.28 ± 0.16
0.5% L-Leucine	356.54 ± 7.83	320.93 ± 15.09	74.13 ± 1.63	89.98 ± 2.43	45.84 ± 9.97	2.92 ± 0.07	2.09 ± 0.01	138.80 ± 32.62	38.86 ± 8.54	11.29 ± 2.76	43.19 ± 9.34	11.35 ± 0.40
1% L-Leucine	394.58 ± 61.56	355.06 ± 58.83	82.03 ± 12.80	89.88 ± 1.58	33.68 ± 9.98	3.01 ± 0.11	2.07 ± 0.06	194.60 ± 61.90	48.45 ± 9.44	11.30 ± 1.67	53.80 ± 9.69	11.76 ± 0.47
5% L-Leucine	386.66 ± 97.37	327.19 ± 109.64	80.39 ± 20.24	84.25 ± 13.76	30.01 ± 4.96	2.91 ± 0.17	2.11 ± 0.06	182.85 ± 73.96	47.19 ± 13.76	17.42 ± 13.92	55.15 ± 8.13	11.42 ± 1.24
10% L-Leucine	376.34 ± 73.37	354.95 ± 68.08	78.24 ± 15.25	94.35 ± 0.64	28.74 ± 9.13	3.20 ± 0.21	2.05 ± 0.05	201.78 ± 58.77	52.96 ± 5.21	9.64 ± 1.01	56.14 ± 5.61	12.14 ± 0.21

Table 6. Content homogeneity of Spray Dried Mannitol, 0.1% $_{L}$-Leucine, 0.5% $_{L}$-Leucine, 1% $_{L}$-Leucine, 5% $_{L}$-Leucine, and 10% $_{L}$-Leucine expressed as the percent coefficient of variation (%CV)

Formulation	Assay (%)	% CV
Spray Dried Mannitol	91.95 ± 19.22	20.90
0.1% L-Leucine	122.37 ± 4.75	3.88
0.5% L-Leucine	105.18 ± 14.81	14.08
1% L-Leucine	112.02 ± 13.38	11.94
5% L-Leucine	110.07 ± 13.11	11.90
10% L-Leucine	110.96 ± 14.84	13.38

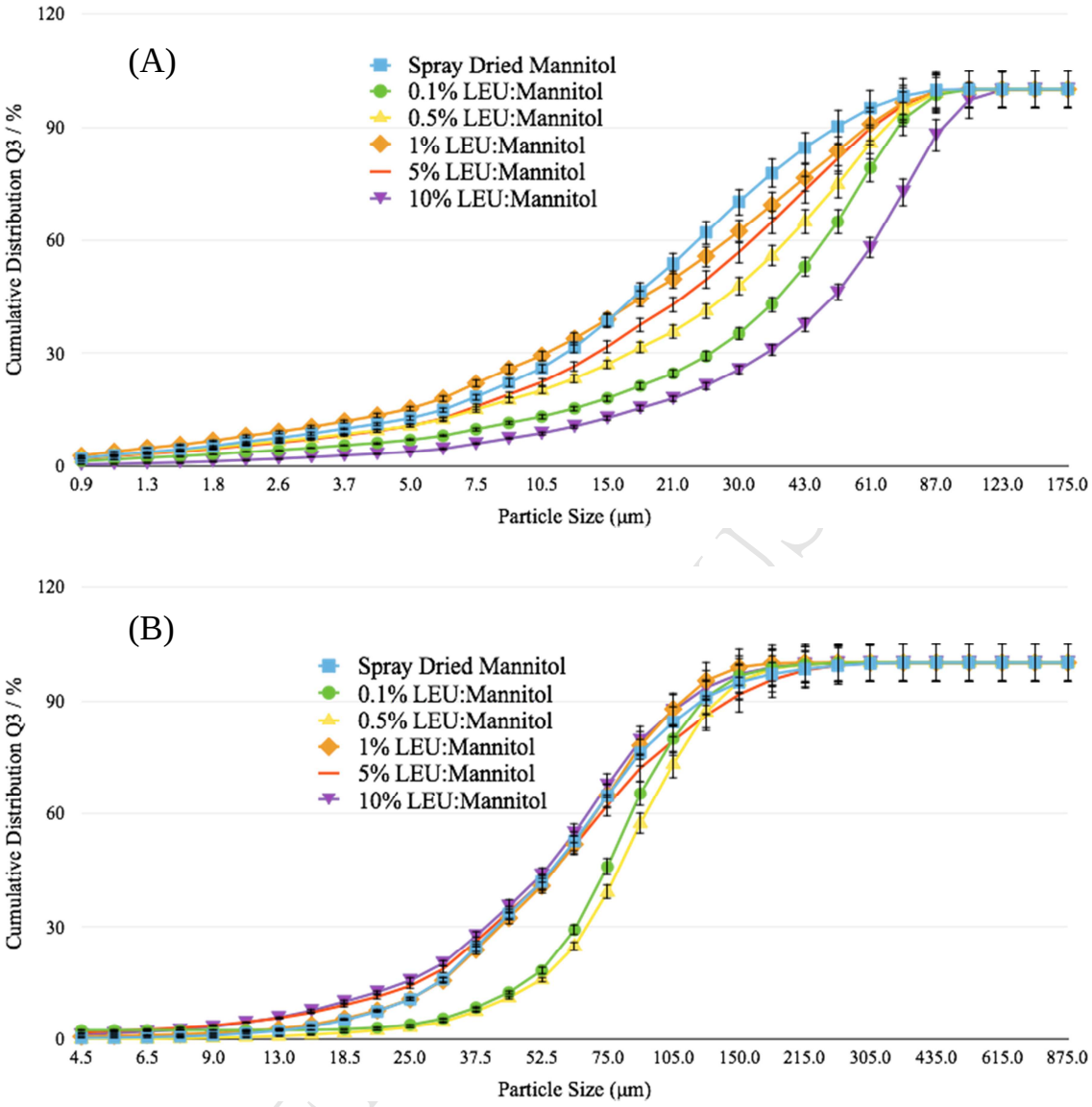


Figure 1. Particle Size Distribution (PSD) diagrams of each formulation's carriers when using the (A) RODOS dry system and when using the (B) Cuvette wet system; Spray Dried Mannitol, spray dried mannitol containing 0.1% $_{L}$-Leucine, 0.1% $_{L}$-Leucine, 0.5% $_{L}$-Leucine, 1% $_{L}$-Leucine, 5% $_{L}$-Leucine, and 10% $_{L}$-Leucine.

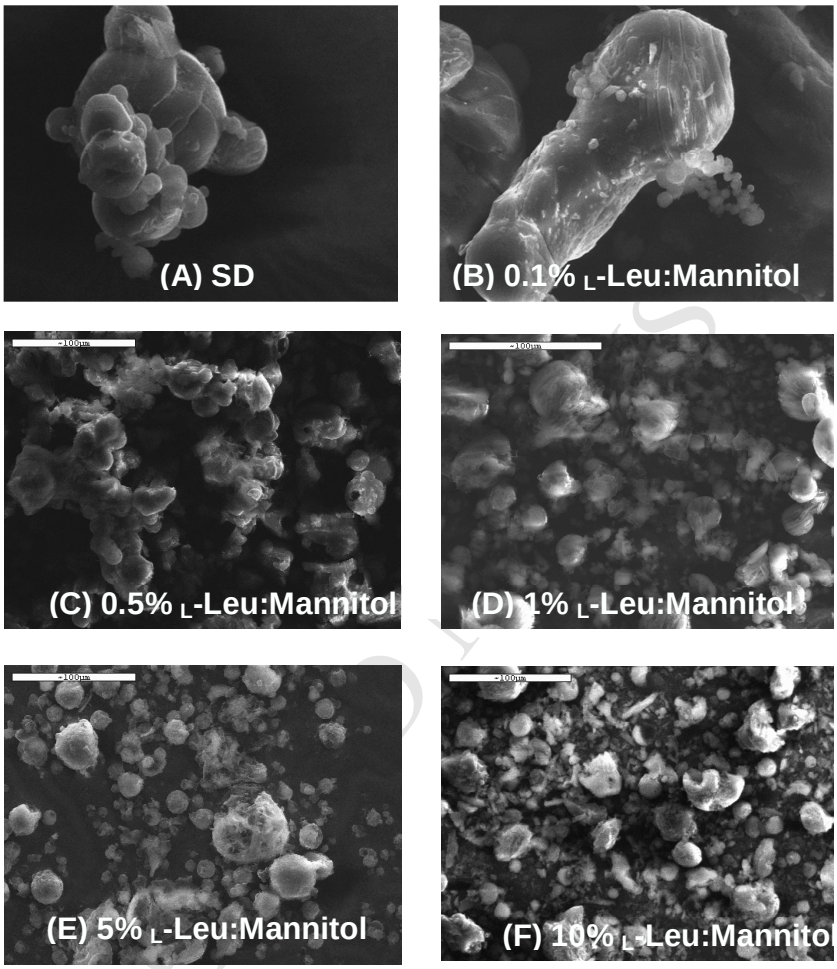


Figure 2. SEM electron micrograms of (A) Spray Dried Mannitol, (B) 0.1% $_{L}$-Leucine, (C) 0.5% $_{L}$-Leucine, (D) 1% $_{L}$-Leucine, (E) 5% $_{L}$-Leucine, and (F) 10% $_{L}$-Leucine.

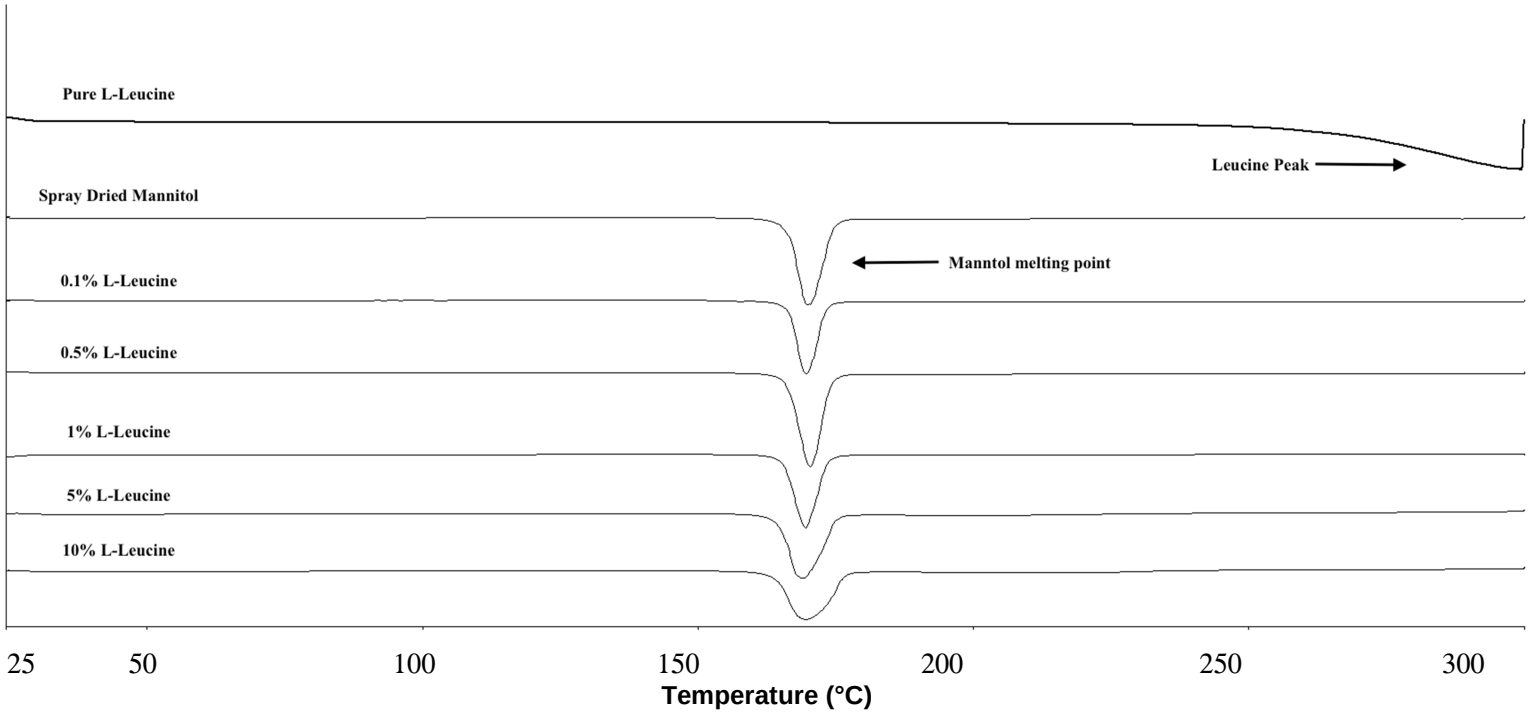


Figure 3. DSC thermal peaks of $_{L}$-Leucine, Spray Dried Mannitol, spray dried mannitol containing 0.1% $_{L}$-Leucine, 0.5% $_{L}$-Leucine, 1% $_{L}$-Leucine, 5% $_{L}$-Leucine, and 10% $_{L}$-Leucine, where an exothermic peak would point up and an endothermic peak would point down.

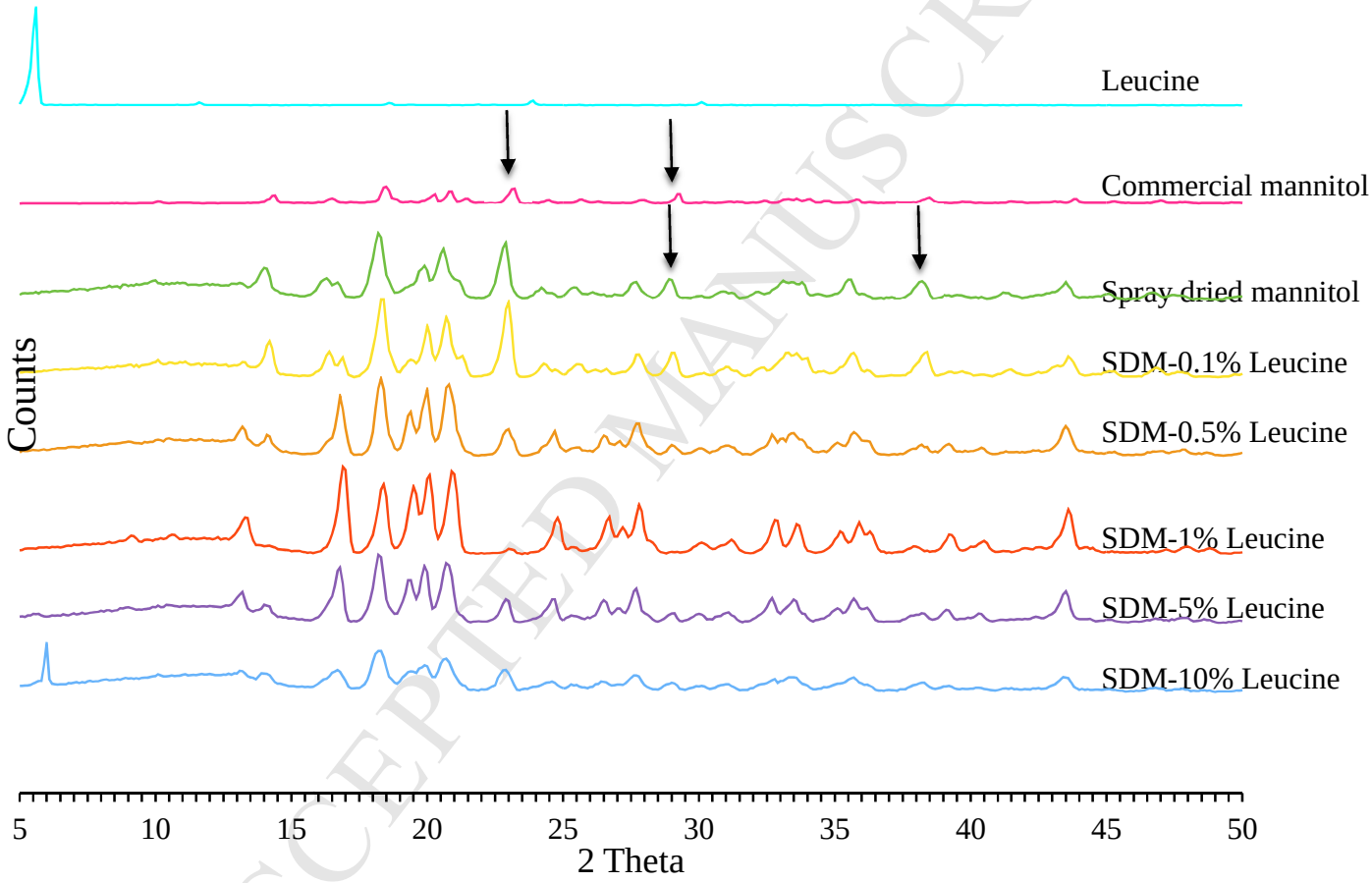


Figure 4. Powder X-Ray diffraction patterns of Leucine, Spray Dried Mannitol (SDM), SDM-0.1% $_{L}$-Leucine, SDM-0.5% $_{L}$-Leucine, SDM-1% $_{L}$-Leucine, SDM-5% $_{L}$-Leucine, and SDM-10% $_{L}$-Leucine.

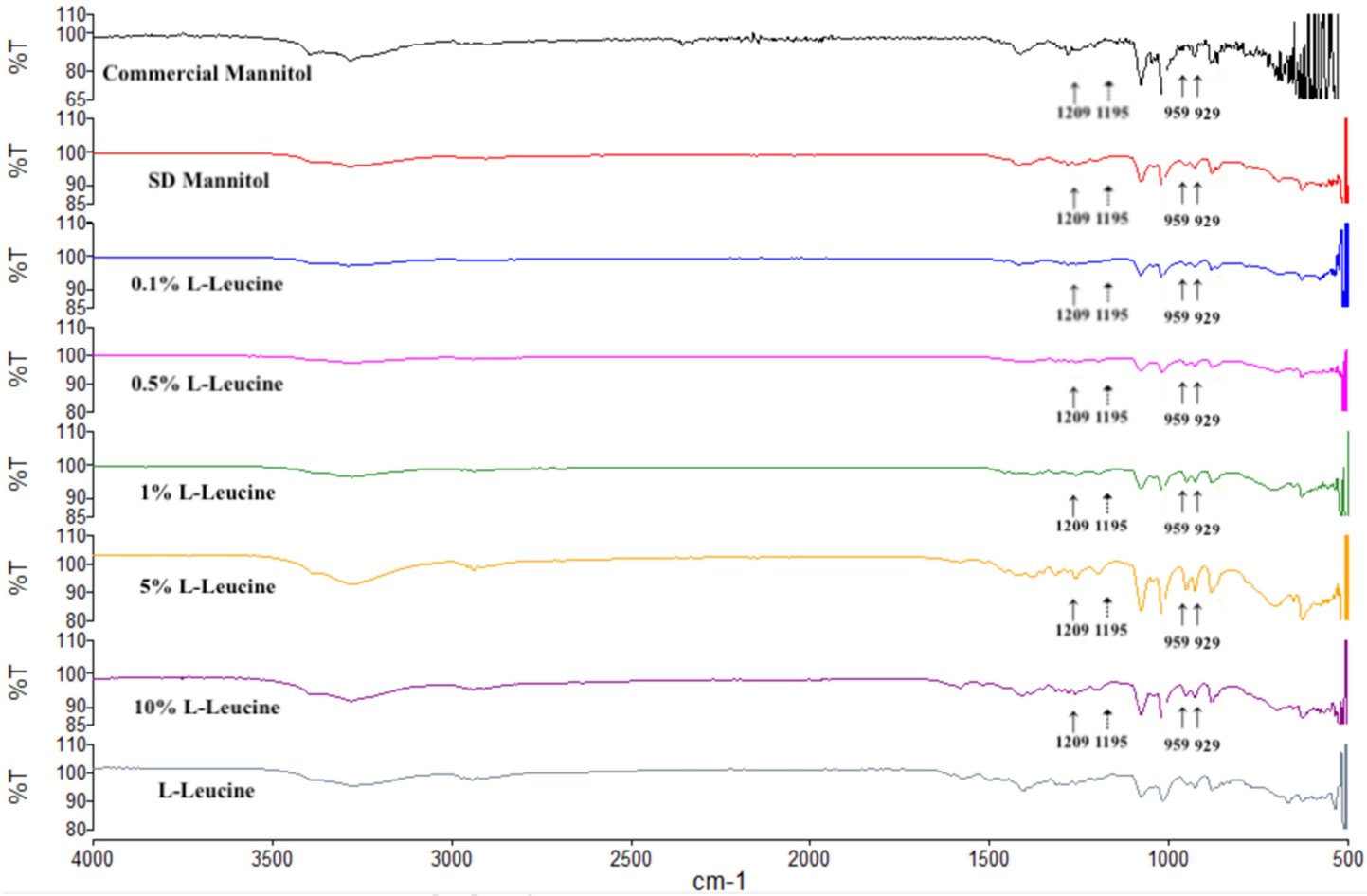
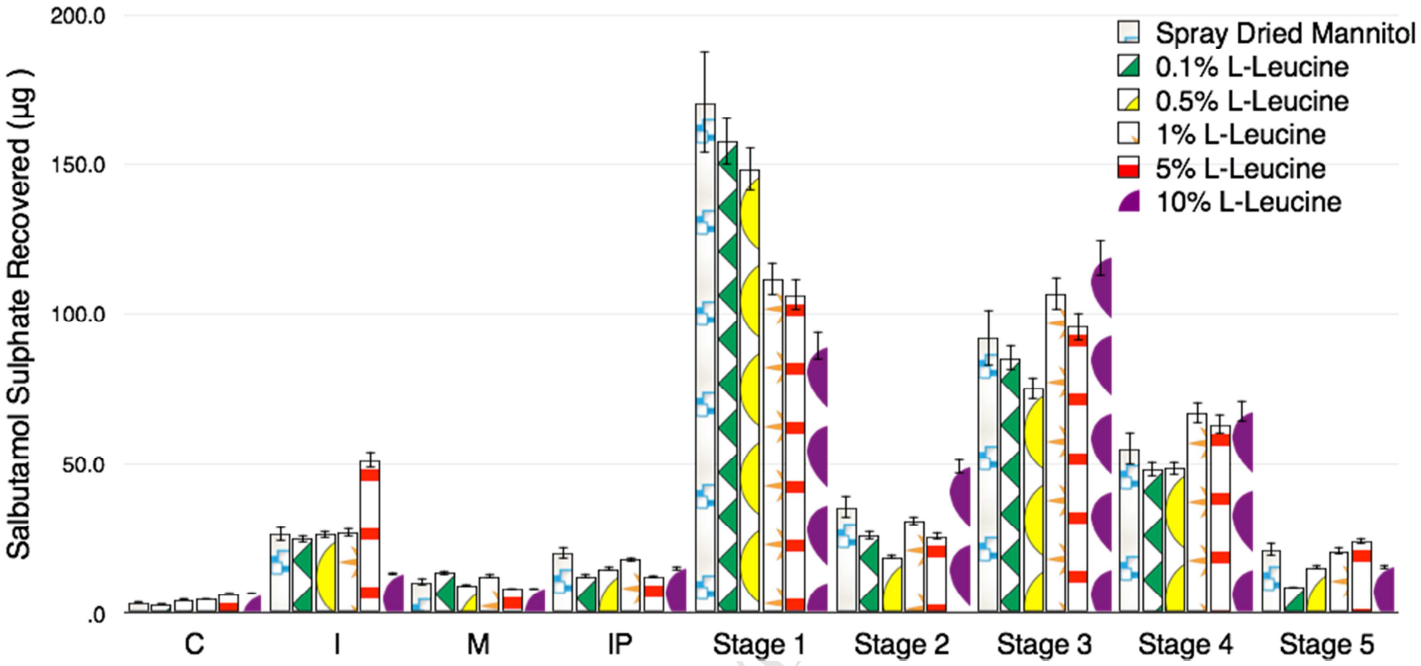


Figure 5. FT-IR spectra of commercial mannitol, Spray Dried Mannitol, spray dried mannitol containing 0.1% $_{L}$-Leucine, 0.5% $_{L}$-Leucine, 1% $_{L}$-Leucine, 5% $_{L}$-Leucine, 10% $_{L}$-Leucine, and $_{L}$-Leucine where ↟ represents α-mannitol, ↑ represents β-mannitol, and ↡ represents δ-mannitol.

Figure 6. Aerosolization performance of each formulation (Spray Dried Mannitol, spray dried



mannitol containing 0.1% $_{L}$-Leucine, 0.5% $_{L}$-Leucine, 1% $_{L}$-Leucine, 5% $_{L}$-Leucine, and 10% $_{L}$-Leucine) highlighting the amount of Albuterol sulfate (AS) recovered (percent recovered).

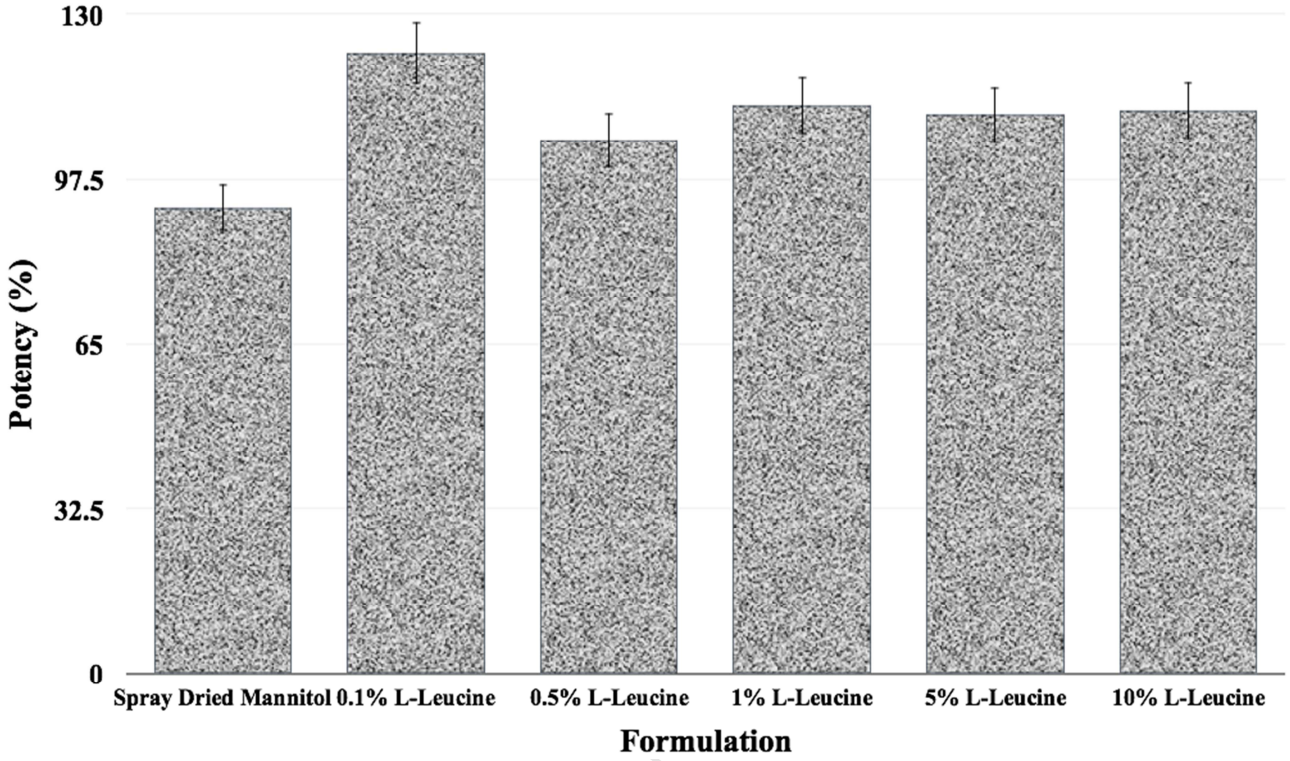


Figure 7. Percent potency of each formulation (Spray Dried Mannitol, spray dried mannitol containing 0.1% $_{L}$-Leucine, 0.5% $_{L}$-Leucine, 1% $_{L}$-Leucine, 5% $_{L}$-Leucine, and 10% $_{L}$-Leucine) with respect to Albuterol sulfate.